

LIMB LOSS AND THE MOLT CYCLE IN THE FRESHWATER SHRIMP, *PALAEMONETES KADIAKENSIS*

LOIS A. STOFFEL AND JERRY H. HUBSCHMAN

Department of Biological Sciences, Wright State University, Dayton, Ohio 45431

In crustaceans, the presence of a chitinous exoskeleton imposes growth limitations on an organism. An animal must molt or shed its exoskeleton periodically in order to grow. Molt cycle control is thought to be regulated by two hormones, a molt-inhibiting hormone and a molting hormone. The X-organs are a group of neurosecretory cell bodies, located near the surface of the medulla terminalis in the crustacean eyestalk, which are presumably responsible for producing the molt-inhibiting hormone (MIH). MIH is then thought to inhibit the Y-organs, the postulated molting glands of crustaceans, from secreting the molting hormone (MH) which acts on the epidermis to initiate premolt or the preparations for ecdysis (Passano, 1960).

Another characteristic of many crustaceans is the ability to regenerate appendages which are lost. Regeneration is dependent upon molting. If a limb is removed at the beginning of the intermolt cycle, a limb bud is produced which grows during premolt (stage D of the intermolt cycle), but not until the animal molts is the limb fully formed and functional. Several molts are required to restore the limb to its original size (Goss, 1969). Since the molting process is under hormonal control, regeneration and normal growth are closely interrelated in crustaceans.

Several studies have been conducted to examine the relationship between regeneration and the molt cycle in crustaceans. Zeleny (1905) noted in three species of crustaceans: the fiddler crab, *Gelasimus* (= *Uca?*) *pugilator*, the pistol shrimp, *Alpheus* and the crayfish, *Cambarus propinquus*, that an excessive loss of limbs led to an increased rate of molting.

Loss of limbs was noted as a molt-accelerating factor for the land crab, *Gecarcinus lateralis* (Bliss, 1956, 1959). Under conditions unfavorable for molting (dry sand, air not saturated with moisture) over a 6-month period, five out of five land crabs missing 6-8 limbs completed premolt limb regeneration and molted, while only one out of nine controls missing 1-2 limbs molted. Skinner and Graham (1970) systematically examined in *Gecarcinus* the effect of loss of limbs on the initiation of molting under favorable conditions. Their results showed that the loss of numerous (6-8) limbs, both walking legs and chelae, caused the organisms to enter proecdysis almost immediately.

Similarly, several species of crabs, including the marsh crab, *Sesarma reticulatum* (Passano and Jyssum, 1963), the green crab, *Carcinus maenas*, the blue crab, *Callinectes sapidus*, and the two fiddler crabs, *Uca pugilator* and *U. pugnax* (Skinner and Graham, 1972), entered proecdysis and molted after induced multiple autotomy.

Two recent reports have mentioned unsuccessful attempts to accelerate the rate of molting by excessive limb loss. Passano and Jyssum (1963) found that

the removal of six walking legs failed to stimulate several large green crabs to enter proecdysis and molt. Also, the spider crab, *Libinia emarginata*, did not respond to limb loss during a ten week observation period (Skinner and Graham, 1972).

The present study was undertaken to determine how the loss of four walking legs affects the duration of the molt cycle of the freshwater shrimp, *Palaemonetes kadiakensis* Rathbun. Previous studies on limb loss have dealt primarily with Reptantia, crustaceans with heavy well-mineralized exoskeletons and adapted for crawling. Except for Zeleny's (1905) preliminary experiment with the pistol shrimp, *Alpheus*, the effect of the removal of several appendages in Natantia, crustaceans lacking well-calcified exoskeletons and adapted for swimming, has not been examined.

Also, in the literature surveyed, only the overall effect of the removal of appendages on molting was noted. Since the intermolt cycle in crustaceans can be subdivided into stages depending upon physiological and morphological changes in the organism (Drach, 1939, 1944; Passano, 1960; Stevenson, 1968, 1972; Stevenson *et al.*, 1968), this paper represents a study of the effect of limb loss at the different stages of the molt cycle.

MATERIALS AND METHODS

The freshwater shrimp used in this investigation were collected from the western end of Sandusky Bay, Lake Erie. At the start of an experiment, each organism was placed in a 4½" Carolina culture dish. The shrimp were fed daily with newly-hatched *Artemia salina* nauplii.

Five groups of organisms were established depending upon the stage (A, B, C or D) of the intermolt cycle in which legs would be amputated, with the controls set up as a separate group. All the experimental organisms were held for five consecutive molts. Between the third and fourth laboratory molts, four walking legs (3rd and 5th pairs) were amputated under 10 × magnification of a dissecting microscope. Using dissecting scissors, each leg was cut between the coxa and the basis. The control organisms were left intact for the five successive molts.

Staging the molt cycle in this investigation was determined according to the methods described by Passano (1960) for *Natantia* which were later modified for *Palaemonetes kadiakensis* (Thompson, 1964). Although the stages can be subdivided in some detail, only the four major stages of the intermolt cycle, A, B, C and D, were considered. Stage A is the stage immediately following ecdysis and is easily distinguished by the hollow appearance of the antennal scale and uropod setae. It was at this stage that the group A shrimp had four walking legs amputated following the third laboratory molt. Stage B occurs when the conical bases of the antennal scale spines are formed. Organisms in group B had four walking legs removed during stage B of their third laboratory intermolt cycle. Stage C occurs when 30% of the intermolt cycle has been completed and no sign of proecdysis is evident. The individuals in group C had four appendages amputated during stage C. Finally, stage D, proecdysis, which occupies 60% of the entire intermolt cycle, is subdivided quite extensively for natantians. The group D shrimp had the amputation of legs performed during late stage D, immediately prior to molt. This stage (D₄) is recognized by the new setae appearing as rods

projecting into the antennal scales and uropods. The development of new setae, upon which recognition of these stages depends, could be seen under a dissecting microscope with $30\times$ magnification.

The laboratory room was maintained on a strict photoperiod of 14 hours of light and 10 hours of darkness which coincided approximately with the normal outdoor photoperiod. Room temperature ranged from 20°C through 26°C , with an average of 24.5°C .

RESULTS

Response to limb loss

In the freshwater shrimp, *Palaemonetes kadiakensis*, the removal of four walking legs caused an acceleration of the molting process (Fig. 1). The average time required for 50% of each group to complete the five laboratory molts (T_{50}) was: group A = 45 days, group B = 44 days, group C = 45 days, group D = 47 days, controls = 52 days. When all of the organisms subjected to limb loss had completed the four intermolt cycles ($T_{100} = 65$ days), only 92% of the controls had finished and it was not until six days later that all the controls had completed the five laboratory molts ($T_{100} = 71$ days). It was observed that on completion of the four intermolt cycles, stage A, stage B and stage C organisms had regenerated the four walking legs, but stage D organisms had not completely regained their limbs.

Response to limb loss during stage A

The amputation of four appendages immediately following a molt leads to the subsequent intermolt period being significantly shortened (Fig. 2). The 120 individuals in group A had a standard mean intermolt cycle of 13.6 days prior to limb loss. The standard mean intermolt cycle is found by taking the mean of intermolt cycles #1 and #2 $((13.4 + 13.8)/2)$. The mean intermolt cycle following limb loss (intermolt cycle #3) was 8.8 days which represents a decrease in intermolt cycle duration of 43.4%. The second postoperative intermolt cycle was 10.4 days. While this was longer in duration than the first, it was still reduced from the standard mean intermolt cycle by 24%.

Confidence intervals for all the means were figured with a 0.99 degree of confidence (Table I). The confidence intervals of the means for the two postoperative intermolt cycles of stage A organisms do not overlap the two preoperative intermolt cycle mean intervals, hence for a 99% degree of confidence the decrease in intermolt cycle duration following limb loss is significant.

Response to limb loss during stage B

The removal of four walking legs during stage B of the third intermolt cycle also caused a shortening of the first postoperative molt (Fig. 2). The molt cycle duration between the third and fourth laboratory molts was reduced by 27%. Also, as in group A shrimp, the second postoperative intermolt cycle was slightly longer in duration than the first postoperative intermolt cycle although it had not returned to normal. Considering that all group B shrimp had four walking legs removed on the second day following the third molt, actually 8% of the intermolt

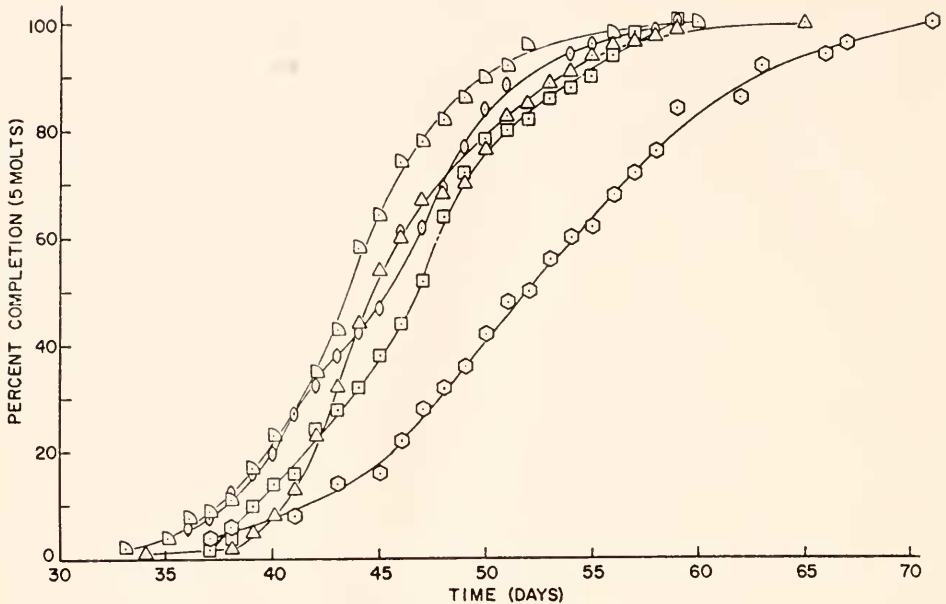


FIGURE 1. Effect of the removal of four walking legs on the duration of four successive intermolt cycles of *Palaemonetes*. All of the organisms were held for two consecutive intermolt cycles before limbs were amputated. During the third intermolt cycle four legs were removed from 120 animals which were in stage A of that intermolt cycle (open triangle), from 51 animals which were in stage B of that intermolt cycle (open arc), from 51 animals which were in stage C of that intermolt cycle (open ellipse), and from 50 animals which were in stage D of that intermolt cycle (open square). The 50 control organisms (open hexagon) were left intact for the five successive molts.

TABLE I
Effect of limb loss on molt cycle duration

Stage	A	B	C	D	X*
Number of organisms	120 (125)	51 (54)	51 (54)	50 (54)	50 (50)
Mean length (mm)	26.3	23.7	23.7	25.0	24.7
Mean intermolt cycle (days \pm S.D.)					
pre-amputation { #1	13.4 \pm 1.9	12.2 \pm 1.9	12.0 \pm 2.0	12.7 \pm 1.5	12.7 \pm 2.2
#2	13.8 \pm 2.3	12.9 \pm 2.2	12.5 \pm 2.2	12.9 \pm 1.9	13.1 \pm 2.2
post-amputation { #3	8.8 \pm 0.9	9.2 \pm 0.9	11.1 \pm 1.4	13.4 \pm 2.2	13.3 \pm 2.4
#4	10.4 \pm 1.4	9.8 \pm 1.4	9.9 \pm 1.4	8.1 \pm 1.1	13.6 \pm 2.6
Confidence intervals for the means (days)					
#1	0.5	0.7	0.7	0.5	0.8
#2	0.5	0.8	0.7	0.7	0.8
#3	0.2	0.3	0.5	0.8	0.9
#4	0.3	0.5	0.5	0.4	1.0
Range (days)					
#1	9-21	9-19	8-15	10-16	8-18
#2	9-19	8-17	9-17	9-16	9-19
#3	7-12	7-11	8-14	9-18	8-19
#4	8-14	7-13	8-13	6-12	9-20

* Controls; brackets indicate initial number of organisms.

cycle had already been completed when limb loss occurred. Thus, the remaining intermolt cycle following amputation was 61% of a standard mean intermolt cycle.

The two postoperative intermolt cycles show significant decreases from the two preoperative intermolt cycles when the confidence intervals of the means are compared.

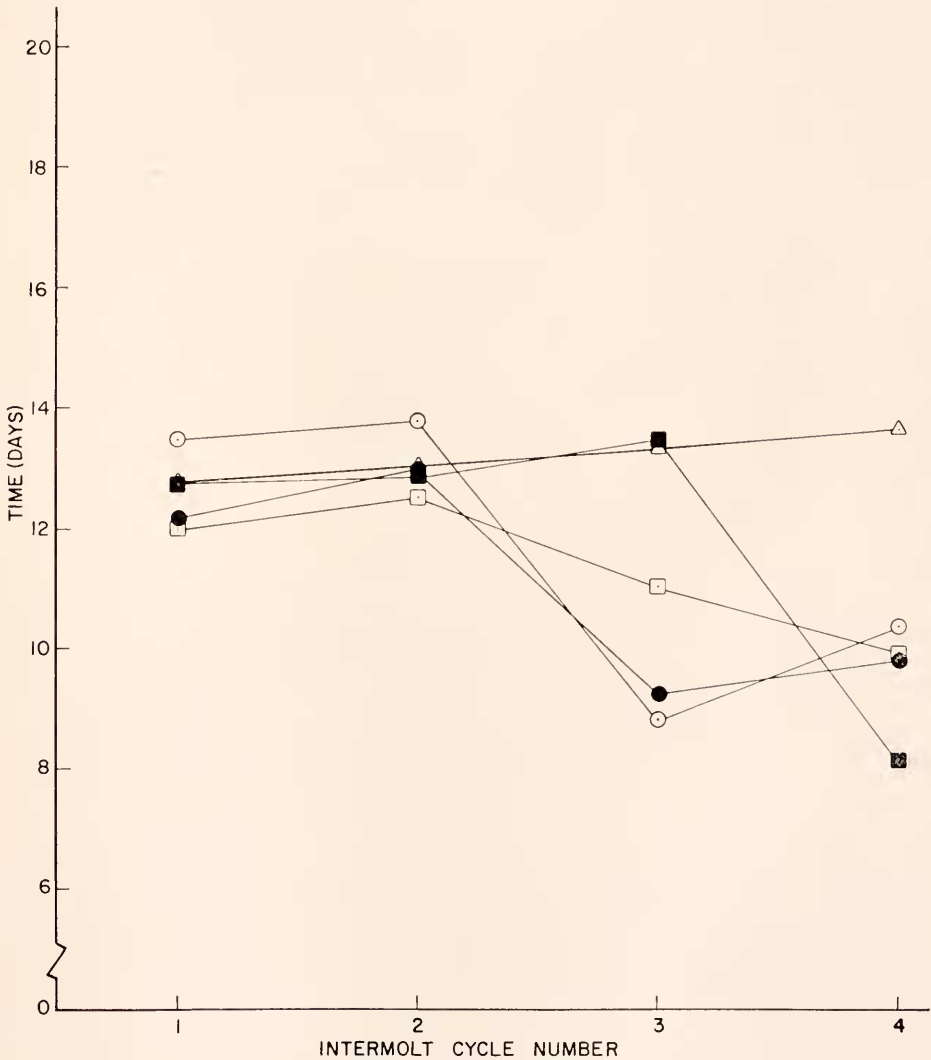


FIGURE 2. Plot of the means of the two preoperative and the two postoperative intermolt cycles of *Palaemonetes*. The group A organisms (open circle) had four walking legs removed during stage A following the third laboratory molt. The group B organisms (closed circle) had four walking legs removed during stage B of the third intermolt cycle. The group C organisms (open square) had four walking legs removed during stage C of the third intermolt cycle. The group D organisms (closed square) had four walking legs removed during stage D of the third intermolt cycle. The control organisms (open triangle) were left intact for four successive intermolt cycles.

Response to limb loss during stage C

Limb loss during stage C of the third intermolt cycle caused a shortening of two postoperative intermolt cycles (Fig. 2). The group C shrimp, which had a mean intermolt cycle of 12.2 days prior to limb loss, showed a slight reduction in the first postoperative molt (9%) and then a further reduction in the second postoperative molt (19%).

Again, if the amount of intermolt cycle completed prior to limb removal is considered, 30% of the entire intermolt cycle had passed before limbs were removed in stage C individuals and thus, the length of the intermolt cycle following amputation was actually 61% of the standard mean intermolt cycle.

As for the confidence intervals of the means, only the second postoperative intermolt cycle was a significant decrease.

Response to limb loss during stage D

When the amputation of four appendages occurred during stage D, the duration of the postoperative intermolt cycle was not affected, but the second intermolt cycle following limb loss was definitely shortened (Fig. 2). The third intermolt cycle of group D organisms showed a 5% increase over the standard mean intermolt cycle. The second postoperative intermolt cycle, however, was reduced by 37% following the removal of appendages.

The decrease of the second postoperative intermolt cycle was a significant decrease over the two preoperative intermolt cycles.

Molt cycle duration in control organisms

The control organisms, those organisms left intact and held for five consecutive molts, had four fairly consistent mean intermolt cycles (Fig. 2). Each molt cycle increased slightly over the previous one: 12.7 days to 13.1 days (+3%) to 13.3 days (+2%) to 13.6 days (+2%).

The confidence intervals of the means show that for a 0.99 degree of confidence there is not a significant difference among the four intermolt cycle durations.

Sex as a factor in limb loss

No difference was noted between males and females with respect to the response to limb loss as both sexes do show that the removal of several appendages does accelerate molting (Table II). The only factor which seems to affect the duration of the intermolt cycle between males and females is the size of the organism as females are larger (26.0 mm) than males (23.7 mm) and therefore, as shown in Table II, have mean intermolt cycles of slightly greater duration.

Mortality

The mortality rate in this investigation was 3.8% (13/337). Death following the removal of four walking legs made up only a small per cent of the total mortality (0.9%), while natural deaths (prior to the third molt) made up the largest per cent of deaths (1.8%). Other reasons for not completing the five laboratory molts included accidental deaths and severe injury resulting from ecdysis.

TABLE II

Comparison of the effect of limb loss between male and female shrimp

	Males	Females
Stage A		
Number of organisms	44	76
Mean length (mm)	24.4	27.4
Mean intermolt cycle (days)		
#1	12.9	13.9
#2	12.4	14.6
#3	8.4	9.1
#4	9.7	10.8
Stage B		
Number of organisms	26	25
Mean length (mm)	23.6	23.8
Mean intermolt cycle (days)		
#1	12.2	12.2
#2	12.8	13.0
#3	8.9	9.6
#4	9.4	10.2
Stage C		
Number of organisms	22	29
Mean length (mm)	22.5	24.7
Mean intermolt cycle (days)		
#1	11.4	12.4
#2	11.6	13.1
#3	10.7	11.4
#4	9.4	10.3
Stage D		
Number of organisms	24	26
Mean length (mm)	24.0	26.0
Mean intermolt cycle (days)		
#1	12.5	12.9
#2	12.2	13.5
#3	12.7	14.1
#4	7.6	8.5
Controls		
Number of organisms	23	27
Mean length (mm)	23.4	25.8
Mean intermolt cycle (days)		
#1	12.3	13.2
#2	12.7	13.3
#3	13.1	13.5
#4	13.0	14.2

DISCUSSION

The experimental results reported here demonstrate that the loss of four walking legs induces molting in the freshwater shrimp, *Palaeomonetes kadiakensis*. They show that regardless of when limb loss occurred during the intermolt cycle, acceleration of molting ultimately resulted. These results support similar findings for several brachyuran species, including the land crab (Skinner and Graham,

1970, 1972); the marsh crab (Passano and Jyssum, 1963); and the green crab, the blue crab and two fiddler crabs (Skinner and Graham, 1972). Consequently, for at least one species of *Natantia*, the response to extensive limb loss is similar to that of *Reptantia*.

The fact that the removal of walking legs did not affect the mortality rate suggests that molts induced by extensive limb loss are normal ecdyses. Skinner and Graham's (1972) work also showed this for *Gecarcinus* with very low mortality rates following ecdysis after limb loss.

In an attempt to explain this molt-accelerating effect of limb loss, Skinner and Graham (1970) discounted the most obvious possible conclusion, that is, that a molt-inhibiting hormone is present in the walking legs. They suggested that the loss of body mass could prevent the secretion of the molt-inhibiting hormone of the X-organs. However, it was shown that the loss of tissue could not be the stimulus responsible for molt initiation since the loss of two chelipeds, which make up approximately 35% of the entire body weight, in the land crab did not accelerate molting while the loss of eight walking legs, which contribute 11–17% of the total body weight, did hasten molting (Skinner and Graham, 1972). Skinner and Graham (1972, page 230) then proposed that "severing of a critical number of nerves" is the stimulus which induces molting.

A possible explanation of the results of the present study is that the loss of several walking legs stimulates the neurosecretory cells of the X-organs via nervous impulses to stop releasing (and also, perhaps, producing) the molt-inhibiting hormone. This would then allow the Y-organs to secrete their postulated molting hormone, and premolt, stage D of the intermolt cycle, would be initiated. In support of this hypothesis it should be noted that the length of the intermolt cycle following limb loss in three stages, A, B and C, closely approximates 60% of the previous intermolt cycle, which is considered the per cent occupied by proecdysis. The effect of limb loss on the molt cycle duration of the first postoperative intermolt cycle in group D individuals was not noted. This is to be expected since the organisms were already in stage D, proecdysis, when amputation of four walking legs occurred. However, if the duration of the second postoperative molt is compared to the previous intermolt cycle, it only amounts to 60.4% of that intermolt period suggesting that excessive limb loss stimulates the organism to enter premolt as soon as possible following the molt.

This hypothesis that stage D is initiated immediately following limb loss seems reasonable, but since molting is a cyclic event, it would seem that stages A, B and C of the intermolt cycle could not be omitted entirely. Hence, the shortening of the intermolt cycle following the removal of appendages could be the result of stages A, B and C being shortened proportionately with the animal molting in the absolute minimal time. Skinner and Graham (1972) did find that eyestalk removal caused land crabs to molt sooner than crabs stimulated to molt by limb removal. However, death usually occurred prior to or at ecdysis in eyestalkless crabs and not in crabs missing appendages. This suggests possibly that the removal of limbs, while stimulating the X-organs to stop releasing MIH, allows the change in concentration from molt-inhibiting hormone to molting hormone to be gradual enough for the organism to hasten through all the stages of the intermolt cycle prior to premolt and thus, a normal molt could occur.

Some explanation can be given for the reports which have noted that the loss of limbs has no effect on molt cycle duration. The most recent mention of inhibition of molting in crustaceans by extensive appendage loss was that the loss of all ten legs in *Gecarcinus* inhibited molting rather than accelerated it (Skinner and Graham, 1972). These crabs, which had lost their limbs in combat with other crabs, showed no sign of regeneration after 90 days in the laboratory. Starvation, which sometimes inhibits molting (Passano, 1960), was not a factor in this situation since the crabs were hand-fed.

As for multiple autotomy failing to induce molting in the green crab, *Carcinus maenas*, Passano and Jyssum (1963) indicated that these crabs were perhaps in terminal anecdyosis (stage C₄T), when molting and growth in the animals have ceased. They described the crabs that they subjected to limb removal as large males and found no signs of approaching molt when they were kept in the laboratory for several months. Skinner and Graham (1972) did find that limb loss induced molting in four green crabs, but they did not mention the size of the individuals. These results suggest possibly that prior to terminal anecdyosis, the removal of several appendages can stimulate molting in *Carcinus maenas*, but once the crab has ceased to molt, extensive limb loss cannot break terminal anecdyosis.

Finally, no molting occurred in the spider crab, *Libinia emarginata*, following the removal of 6-8 walking legs. None of the individuals, including eyestalkless and control organisms, molted in the laboratory during the ten week observation period (Skinner and Graham, 1972). Since the organisms were held in community tanks during the experiment, it was suggested that perhaps in this organism, as in *Gecarcinus* (Bliss and Boyer, 1964), privacy at the time of molt is a "critical factor" which prevents crabs from molting in the presence of another crab. In this sense, molting would be prevented by the lack of privacy overriding any stimulus to molt from limb loss.

SUMMARY

1. The loss of four walking legs induces molting in the freshwater shrimp, *Palaeomonetes kadiakensis*. This study shows that at least in one species of *Natantia*, excessive limb loss accelerates the molting process as has been shown in several reptantian species.

2. Removal of four appendages (two pairs of walking legs) during the four major stages of the molt cycle, A, B, C and D, produced varying results. When the appendages were removed during stage A or stage B of the intermolt cycle, the first molt following limb loss was accelerated. When limbs were removed during stage C of the molt cycle, two successive molts following limb loss were accelerated. Finally, when limb removal occurred during stage D of the intermolt cycle, the first postoperative molt was not affected, but the second postoperative intermolt cycle was shortened significantly.

3. The sex of the organism did not affect the response to the loss of numerous appendages.

4. Mortality rates due to the loss of four walking legs were negligible.

5. A hypothesis was proposed to explain this molt-accelerating effect of limb loss.

LITERATURE CITED

- BLISS, D. E., 1956. Neurosecretion and the control of growth in a decapod crustacean. Pages 56-75 in K. G. Wingstrand, Ed., *Bertil Hanstrom: Zoological Papers in Honour of his Sixty-fifth Birthday*, November 20, 1956. Zoological Institute, Lund, Sweden.
- BLISS, D. E., 1959. Factors controlling regeneration of legs and molting in land crabs. Pages 131-164 in F. L. Campbell, Ed., *Physiology of Insect Development*. University of Chicago Press, Chicago.
- BLISS, D. E., AND J. R. BOYER, 1964. Environmental regulation of growth in the decapod crustacean, *Gecarcinus lateralis*. *Gen. and Comp. Endocrinol.*, **4**: 15-41.
- DRACH, P., 1939. Mue et cycle d'intermue chez les Crustacés Décapodes. *Ann. Inst. Oceanogr. Monaco*, **19**: 103-391.
- DRACH, P., 1944. Etude préliminaire sur le cycle d'intermue et son conditionnement hormonal chez *Leander serratus* (Pennant). *Bull. Biol. France Belg.*, **78**: 40-62.
- GOSS, R. J., 1969. *Principles of Regeneration*. Academic Press, New York and London, 287 pages.
- PASSANO, L. M., 1960. Molting and its control. Pages 473-536 in T. H. Waterman, Ed., *The Physiology of Crustacea. I. Metabolism and Growth*. Academic Press, New York and London.
- PASSANO, L. M., AND S. JYSSUM, 1963. The role of the Y-organ in crab proecdysis and limb regeneration. *Comp. Biochem. Physiol.*, **9**: 195-213.
- SKINNER, D. M., AND D. E. GRAHAM, 1970. Molting in land crabs: stimulation by leg removal. *Science*, **169**: 383-385.
- SKINNER, D. M., AND D. E. GRAHAM, 1972. Loss of limbs as a stimulus to ecdysis in Brachyura (true crabs). *Biol. Bull.*, **143**: 222-233.
- STEVENSON, J. R., 1968. Metecdysial molt staging and changes in the cuticle in the crayfish *Orconectes sanborni* (Faxon). *Crustaceana*, **14**: 169-177.
- STEVENSON, J. R., 1972. Changing activities of the crustacean epidermis during the molting cycle. *Amer. Zool.*, **12**: 373-380.
- STEVENSON, J. R., R. H. GUCKERT AND J. D. COHEN, 1968. Lack of correlation of some proecdysial growth and developmental processes in the crayfish. *Biol. Bull.*, **134**: 160-175.
- THOMPSON, E. M., 1964. Gross external changes of the adult freshwater prawn, *Palaeomonetes kadiakensis*, during the molting cycle. *M.S. thesis, Ohio State University*, 18 pages.
- ZELENY, C., 1905. Compensatory regulation. *J. Exp. Zool.*, **2**: 1-102.